SEMI-ANNUAL STATUS REPORT

NASA GRANT NGR-39-002-011
April 1, 1965 - September 30, 1965

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The development of microspectrophotometry and the necessary instrumentation to identify organic molecules in studying the chemistry of living cells has progressed to the extent that there are now several functioning instruments in our laboratory. The instrumentation was begun in 1960; these microspectrophotometers have been designated as M-1, M-2, M-3, and M-4. The design and performance of these instruments have been presented in various reports and publications (1-6).

Based on our experience, a completely new instrument is now being constructed in our experimental shop. The M-3 and M-4 microspectrophotometers now in use in the laboratory are limited in their response by the electronics and by the type of recorder used. A new instrument, M-5, now under construction will use a recorder with a D'Arsonval-type pen drive rather than the servomechanism. Also, the electronics will be much more versatile in response speeds. The new recorder and electronics will permit scanning the spectrum, 400 to 700 mμ, in a minimum time of ten seconds as compared with the time of approximately two to four minutes for the same part of the spectrum.

The main improvement in this instrument has been in sensitivity by using an EMI 9558QA photomultiplier tube which has a spectral response from 162 mμ to 840 mμ, with a quantum efficiency of about 20% from 200 to 420 mμ, 10% efficiency at 480 mμ, and 1% efficiency at 785 mμ. The dark current noise is extremely low and with cooling (-12°C) is almost negligible. The level of light will be limited to this dark current noise. A Canalco rapid-scanning monochromator, which was purchased for this purpose, functions well in the instrument. This monochromator has a 600 line/mm grating and has ten different speeds from two seconds to 1000 seconds over 200 to 800 mμ. Its dispersion is 4 mμ/mm and peaks at 500 mμ. The light is about 10 to 20 per cent polarized, which is a disadvantage found in all grating monochromators. A quartz diffusing plate, which can be used, cuts down the polarization.

The accuracy in measuring the relative percentage of absorption as far as electronics are concerned primarily depends on the relative heights of the two pulses, the sample and reference, in the pulse pair. The light from the sample and reference areas is not measured simultaneously but alternately in time, because a single photomultiplier is employed. Therefore, if the overall accuracy is to stay within one per cent, any change in the relative height of the pulses due to relative spectral quantum efficiency of the photocathode, the relative spectral distribution of the light source, or the gain of the amplification should be within one per cent during the time for one pair of pulses. Consequently, a short-scanning run-time requires a correspondingly short pulse pair-time. The desired short pulse pair-time required the development of a precision, high-speed chopper and special electronic clamping circuits in the amplifier. A precision chopper constructed in the laboratory will chop the light at 480 cps.

In order to view specimens which are photosensitive, special techniques are necessary. A cold stage on the microscope helps reduce thermal "bleaching" effects. Another is the use of an infra-red converter, which reduces the bleaching; however, it also reduces the optical resolution.

In order to overcome these inadequacies we are investigating the image intensifier which allows us to view specimens under very low light levels of illumination and with increased optical resolution. A part of these investigations are being carried on in collaboration with Professor George T. Reynolds in the high energy physics laboratory at Princeton University.

A schematic of the microspectrophotometer, M-5, is illustrated in Figure 1. The adaptability of this instrument to space exploration has also been considered in its design. This adaptability includes performance, specimen handling, and focusing.

MICROSPECTROPHOTOMETER M-5

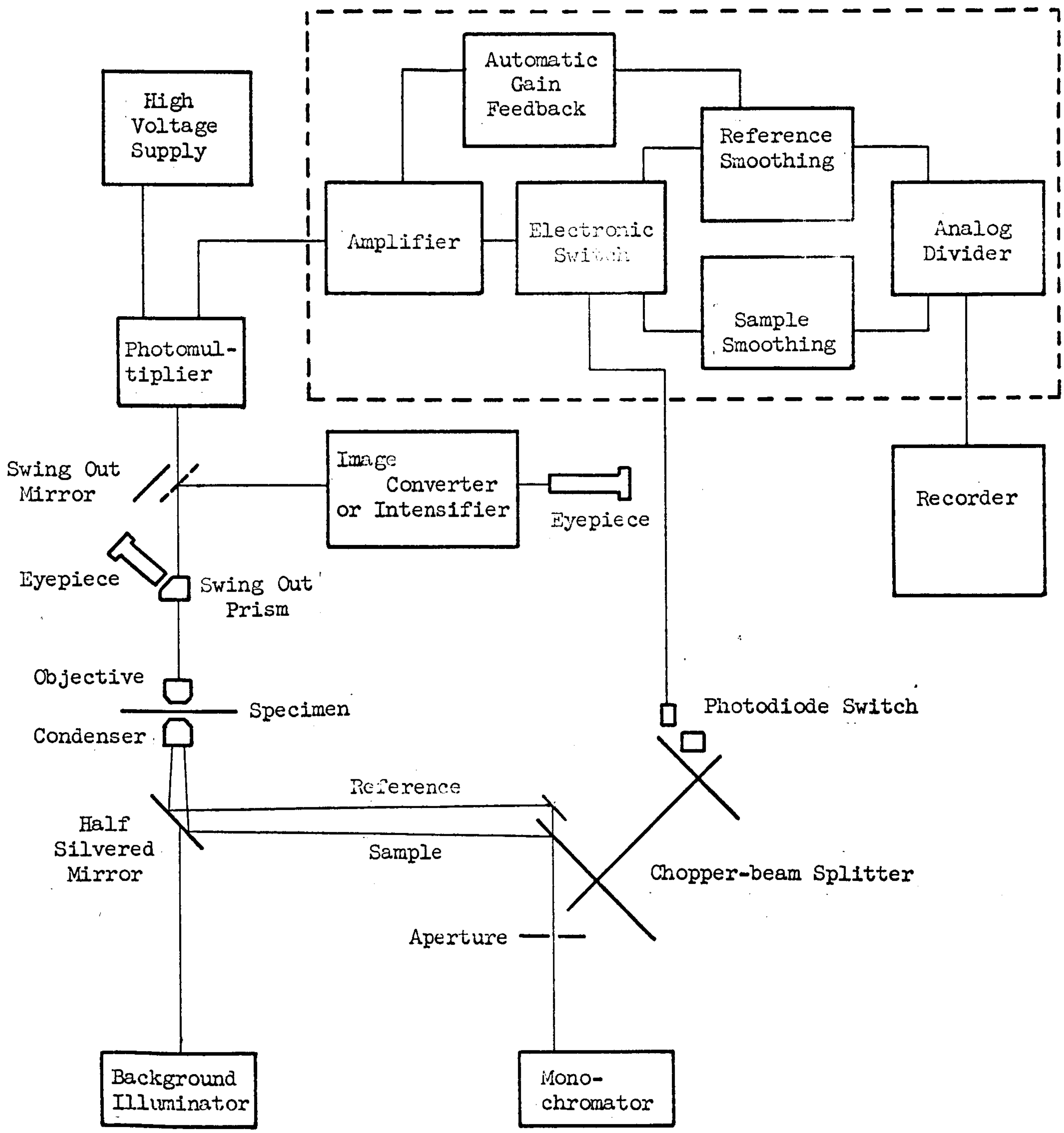

Figure 1

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RELATED PUBLICATIONS

1. Wolken, J. J. and G. K. Strother, "Microspectrophotometry," Applied Optics, 2(9): 899, 1963.

2. Wolken, J. J. Tenth Annual Report of the Biophysical Research Laboratory, Eye and Ear Hospital, University of Pittsburgh School of Medicine, and Carnegie Institute of Technology, Pittsburgh, Pennsylvania, 1964.

3. Wolken, J. J., "Photoreceptor Structures and Energy Transfer," J. Ark. Med. Soc., 62: 61, 1965.

4. Wolken, J. J. Vision: Biophysics and Biochemistry of the Retinal Photoreceptors. Charles C. Thomas, Publisher, Springfield, Illinois, 1965.

5. Wolken, J. J., "A Simplified Microspectrophotometer," Encyclopedia of Spectroscopy, G. L. Clark, ed., Reinhold Publishing Corporation (in press, 1965).

6. Wolken, J. J., "Lipids and the Molecular Structure of Photoreceptors," abstract published in the proceedings of the American Oil Chemists Society Symposium, October 12, 1965, Cincinnati, Ohio. Complete paper to be published in proceedings of "Metabolic Roles of Lipids" symposium.